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STR Typing of Human DNA from Fly Larvae Fed on Decomposing Bodies*

ABSTRACT: In homicides with entomological evidence, it may be important to prove the presumed association of fly larvae to a corpse, especially if it is in doubt whether all maggots used for entomological expertise developed and fed on it. The present study demonstrates for the first time the possibility of analyzing human microsatellite DNA present in the digestive tract of necrophagous larvae that fed on decomposed bodies with a postmortem interval up to four months. The obtained human STR profiles support the association of a maggot to a specific corpse. In addition, the identification of the host species (e.g., animal source like pig) can be achieved by analysis of the cytochrome b gene.

Maggots were collected from 13 corpses after various postmortem intervals and STR typing and HVR amplifications were performed using their crop contents. In seven cases, a complete STR profile was established, in two cases, an incomplete set of alleles was obtained, and in four cases, STR typing was not successful. HVR analysis was successful in all cases except one. The time of storage of the maggots and the length of the postmortem interval up to 16 weeks appeared to have no particular influence on the quality of the results.

KEYWORDS: forensic science, forensic entomology, STR typing, hypervariable regions, crop content, maggots

Investigation of insect larvae found on human corpses is an important method for estimating the postmortem interval (PMI). Immature stages, mostly fly maggots, are collected from and around the corpse at a crime scene. PMI is then calculated by determining the insect species and estimating their stage of development (1–4). However, in certain cases, it may be important to prove the association of specific maggots to a particular corpse. For example, maggots but no corpse may be discovered, indicating that a dead animal was removed from the scene. The determination that this was a human corpse would immediately trigger a criminal investigation. Furthermore, analysis would be helpful when it is not clear whether all maggots used for entomological expertise developed and fed on the particular corpse. This question could arise when the maggots from one crime scene were divided, sent to different investigators and inconsistent conclusions were taken from the same evidence (5, Haskell NH, St. Joseph's College, Rensselaer, IN. Personal communication).

Under these circumstances, DNA analysis of human material ingested by the maggot could yield a genetic profile suitable for comparison to that of a corpse. During the process of digestion, the liquefied host tissue is stored temporarily in a special region of the maggot's foregut, the crop. Therefore, for allocation of maggots to a specific corpse, examination of the crop content with typing methods commonly used in human DNA analysis such as STR typing (6) or evaluation of a hypervariable region (HVR) within the mitochondrial d-loop sequence (7,8) is reasonable.

It has been demonstrated that it is possible to obtain mtDNA-, AMPFLP- and STR-data from adult insect blood meals that can lead to individualization of the ingested host blood (9–11). Laboratory studies have also shown that the contents of a maggot crop can be used to obtain a human or non-human mtDNA haplotype (5,14) or a human Y-STR profile (12). Also, human HVR analysis using beetle larvae that fed on human bone is possible (13). These authors presented arguments for the potential forensic utility of carrion insect gut analysis, and they demonstrated that such an analysis can be successful under carefully controlled conditions and using relatively sensitive mtDNA techniques and/or undegraded tissue. It is still not certain, however, if a less sensitive but more informative STR analysis is likely to work given the typically degraded state of a human corpse from an actual death investigation.

In this study, the crop contents of maggots collected from human corpses with different PMI was subjected to STR and, for comparison, HVR genetic analysis.

Material and Methods

Samples

Feeding third instar larvae were collected from 13 human corpses during autopsy at the Institute of Legal Medicine, Frankfurt/M., Germany from December 2001 until August 2002. Specimens included blowflies (Diptera: Calliphoridae) from ten corpses, flesh flies (Diptera: Sarcophagidae) from two corpses and houseflies (Diptera: Muscidae) from one corpse. The maggots were killed in boiling water and stored in 70% ethanol for one day to two months prior to analysis. Tissue samples from the 13 corpses were also collected and stored at –20°C prior to examination. All corpses showed marked signs of putrefaction ranging from released rigor mortis to advanced and greenish colored decay. Except for three bodies, all cadavers were found indoors. The postmortem interval of the corpses was estimated by police investigations. Calliphorid maggots used to demonstrate the identification of non-human DNA were collected from a pig carcass.

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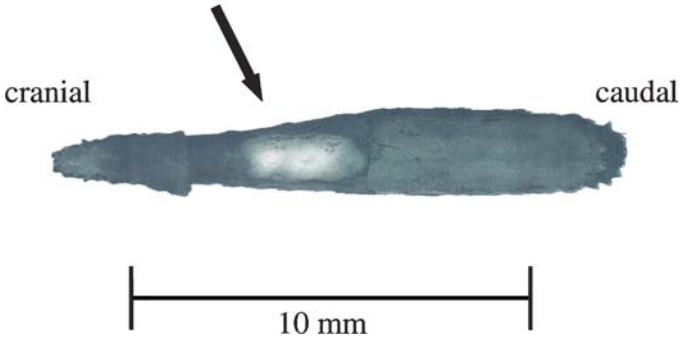

FIG. 1—*Crop situs (arrow) with* Calliphora *larva (dorsal view), colors inverted. Ethanol sample. Magnification: 5×, light microscope Nikon SMZ-U.*

Crop Dissection

Maggots were opened dorsally with a scalpel under a binocular microscope to excise the crop. The sizes of the crops ranged from 1 to 3 mm (Fig. 1). Initially, genetic analysis was attempted on a single larva from each corpse. If STR amplification from that larva was unsuccessful, up to four additional maggots from the same corpse were analyzed.

Human DNA Analysis

DNA extraction was performed using standard phenol-chloroform extraction and ethanol precipitation (15). The DNA of one crop was dissolved in 25μL of water. STR-PCR was carried out with 10 μL of DNA extracts from crop contents and corpse tissue using the commercially available multiplex kit "Profiler Plus" (Applied Biosystems, Foster City, CA) according to the manufacturer's recommendations, but 32 cycles instead of 29 cycles were used to amplify the DNA of the crop content. Capillary electrophoresis was performed in an ABI PRISM 3100 genetic analyzer (Applied Biosystems, Foster City, CA) using a set of 36 cm capillaries with a diameter of 50 μm, POP4, and the standard running module "Genescan36_POP4" as recommended by the manufacturer. STR typing was done using genescan analysis software version 3.7 (Applied Biosystems, Foster City, CA). To get information about the presence of human DNA even in samples which may fail in STR typing, also the mitochondrial d-loop was amplified that represents a more sensitive method than STR typing. We used the primer pair F15971/R16410 for HV I as decribed in (16) and the primer pair L48/H408 for HV II as described in (17) for PCR without further sequence analysis. Amplicons were visualized after agarose-gelelectrophoresis and ethidiumbromide-staining under UV light.

Species Specific DNA Analysis

DNA samples from the crop material of other maggots which were feeding on a pig carcass were amplified in a PCR reaction using cytochrom b specific PCR, resulting in a 307 bp fragment. The cytochrome b gene reveals species specific sequences, by comparison of questioned sequences with reference sequences the origin of the questioned sample can be deduced. Identical sequences point at identical species (18). Sequence determination of the PCR products was performed using the PCR primers separately in a cycle sequencing reaction (Big Dye Terminator Cycle Sequencing Kit, Applied Biosystems, Foster City, CA) according to the manufacturers recommendations. Electrophoretic separation was performed using an ABI 310 Prism genetic analyzer (Applied Biosystems, Foster City, CA). Sequence analysis was performed using Sequence analysis version 2.1.2 and Sequence Navigator software version 1.0.1 (both Applied Biosystems, Foster City, CA).

Results and Discussion

All corpse tissues yielded complete genotyping data (data not shown). In most cases, STR typing of the crop content from maggots was successful. In seven cases complete STR profiles (i.e., 10 loci) were obtained. In two cases incomplete STR profiles (<10 loci and/or allelic drop-out) were observed. In four cases STR typing failed.

STR profiles obtained from the crop content matched the profile of the corresponding corpse in all cases (Fig. 2). DNA from all body samples and all crop samples except those from body No. 12, yielded amplicons of HV 1 and HV 2 (data not shown).

From this study, it can be concluded that STR analysis of a maggot's crop content can be used to associate particular maggots to a given corpse of forensic interest. Because crop-content DNA is likely to be degraded, 32 PCR cycles are recommended, although in some cases (Fig. 2), the obtained data peaks were high enough to suggest fewer cycles.

Successful STR typing of a maggot's crop content can be achieved even after a PMI of about four months, as corpse No. 13 indicates (Table 1). This person had died in autumn and the corpse was found

TABLE 1—*Results of the STR-typing using the crop content of Diptera maggots, which fed on corpses with different postmortem intervals. All habitats were indoor scenes (apartments) with the exception of:* a *(open hen-coop),* b *(forest),* c *(caravan). Probable time of death resp. PMI was evaluated by criminal investigations.*

No. Corpse	Probable Month of Death	Probable PMI (Weeks)	Diptera	Maggot Storage Prior Dissection	STR
1	August	1	CA	<1 week	no
2	May	1,5	CA	2 months	yes
3	June	1,5	CA	2 months	yes
4	July	1,5	CA	2 months	no
5	May/June	2,5	SA	2 months	yes
6[a]	July	2,5	CA	<1 week	incomplete
7	July	3	SA	1 day	no
8	May	4	CA	2 months	yes
9	July	4	CA	<1 week	yes
10	January	6	CA	<1 week	yes
11	May	10	CA	1 day	incomplete
12[b]	December	14	CA	<1 week	no
13[c]	December	16	MU	<1 week	yes

[a]Diptera: CA = Calliphoridae; [b]MU = Muscidae; [c]SA = Sarcophagidae.

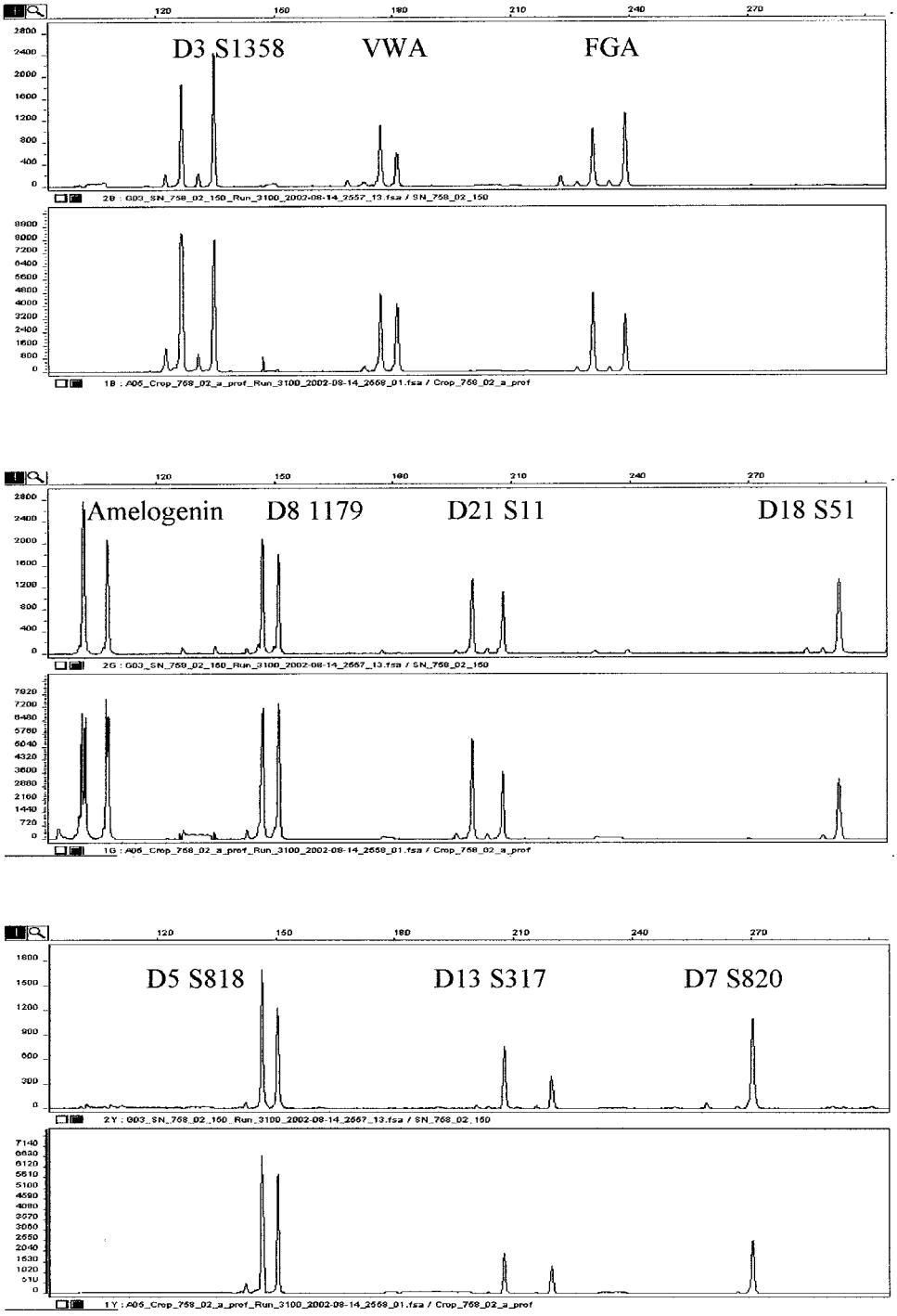

FIG. 2—*Typical electropherograms of Profiler + loci from crop-DNA of a maggot (below) and of the corresponding corpse-DNA (above).*

in a caravan in a highly mummified state. The cold climate conditions during winter may have prevented the DNA degradation in the soft tissue and therefore successful STR typing was possible. However, as seen in corpse No. 12 (no STR profile and no HVR amplicons from the crop sample, PMI 14 weeks) other parameters also appear to influence the success of DNA typing results. Biochemical alterations during food digestion from the maggot may represent a factor for further degradation, leading to a failure of typing from the crop material. Primarily digestion does not occur within the crop, because proteolytic enzymes are not secreted into this area of the foregut, which mainly acts as a food container. However, enzymes are present in the saliva of the maggot for pre-oral digestion and are re-incorporated with the food into the crop (19,20). Therefore, degradation of DNA may occur even within the crop, but the extent of degradation may vary and predictions about the time period during which STR typing can be successfully performed are difficult to evaluate. Because of the limited sample size in this study, further observations are needed before we can understand when crop analysis is likely to succeed or fail.

In cases where STR typing is not possible due to degraded DNA or too low amounts of target molecules, for example, in telogen or rootless hair, heavily putrefied tissue, bone or faeces (7,21–23), the

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Crop content CTATCTACAT GCAAACGGAG CATCCATATT CTTTATTTGC CTATTCATCC ACGTAGGCCG
S. scrofa    .......... .......... .......... .......... .......... ..........
H. sapiens   ...C..T..C ..C..T..C. .C..A..... ......C... ..C...C.A. ..A.C..G..
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FIG. 3—*Partial sequence of the cytochrome b gene, (pos. 15676–15735 of the pig, genebank NC 000845,* Sus scrofa*) the human sequence, (genebank X93334) and of the crop material. Dots represent identical bases compared to the sequence of the crop content of larvae after feeding on a pig carcass.*

analysis of human mtDNA sequences is an alternative method. This strategy is applicable also in case of examination of crop content.

In nearly every DNA sample from the investigated crops, including the samples that failed to generate STR profiles, HV I and HV II amplicons of about 300 bp could be generated. This suggests that in many cases mitochondrial sequences can be analyzed, although STR profiles were not obtained. With the crop content of the maggots that fed on corpse No. 12, neither STR typing nor HVR amplification was possible although HVR I and II amplicons were obtained from the material of the body. Thus DNA analysis may be reduced due to degradation during ingestion.

When neither STR nor HVR data can be obtained in maggots collected at a crime scene, it also has to be taken into consideration that these maggots may have fed on other than human material, e.g., on human food such as pork, beef, chicken etc. in the flat where the corpse was found or supposed to have been previously. This assumption can be verified by species specific sequence-analysis of the cytochrome b gene from the crop content and comparison with reference samples. A sequence that is characteristic for nonhumans points at another than a human food, which could be demonstrated by evaluation of the crop content from maggots which fed on a pig carcass. Species analysis was successful in crops of these maggots, the sequence of the cytochrome b fragment matched the pig reference sequence (*Sus scrofa*; GeneBank NC 000845, Fig. 3).

Storage time in 70% ethanol does not seem to be critical within the periods investigated. Complete STR profiles could be obtained even after two months of storage (Corpses No. 2, 3, 5 and 8) confirming the fact that ethanol is a useful preservative for tissue that has to be analyzed for DNA (24,25).

The taxon of the maggots, which are analyzed for STR-typing, appears to have no influence on the results. Complete STR profiles could be obtained regardless of the Diptera family. It is remarkable that even analysis of the crop content of flesh flies (Diptera: Sarcophagidae), which are known to be predaceous on other maggots during their last larval stages, resulted in a complete human STR-profile. This indicates that these flies were still necrophagous or at least schizophagous, feeding on dead tissue and preying on other necrophagous maggots as well.

After ingestion ceases, the crop will be emptied. Therefore further investigations are necessary concerning the time period during which STR typing of crop extracts can be successful after the maggot had left or had been removed from the corpse. These data are neccessary particularly in cases where human genotyping fails. In these cases, it is important to evaluate whether negative results indicate that no human food source was present or simply that the maggot was no longer suitable for the analysis.

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